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Preservation and promotion of bone formation in the mandible as a response to a novel calcium-phosphate based biomaterial in mineral deficiency induced low bone mass male versus female rats

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Abstract

Calcium and other trace mineral supplements have previously demonstrated to safely improve bone quality. We hypothesize that our novel calcium-phosphate based biomaterial (SBM) preserves and promotes mandibular bone formation in male and female rats on mineral deficient diet (MD). Sixty Sprague-Dawley rats were randomly assigned to receive one of three diets ($n = 10$): basic diet (BD), MD or mineral deficient diet with 2% SBM. Rats were sacrificed after 6 months. Micro-Computed Tomography (μ CT) was used to evaluate bone volume and 3D-microarchitecture while microradiography (Faxitron) was used to measure bone mineral density from different sections of the mandible. Results showed that bone quality varied with region, gender and diet. MD reduced bone mineral density (BMD) and volume and increased porosity. SBM preserved BMD and bone mineral content (BMC) in the alveolar bone and condyle in both genders. In the alveolar crest and mandibular body, while preserving more bone in males, SBM also significantly supplemented female bone. Results indicate that mineral deficiency leads to low bone mass in skeletally immature rats, comparatively more in males. Furthermore, SBM administered as a dietary supplement was effective in preventing mandibular bone loss in all subjects. This study suggests that the SBM preparation has potential use in minimizing low peak bone mass induced by mineral deficiency and related bone loss irrespective of gender.

Keywords

Calcium phosphate biomaterial; mineral deficiency; peak bone mass; animal studies; bone μ CT; microradiography

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

INTRODUCTION

Studies indicate that approximately 54% of the US adult population, aged 50 years or older, were affected by low bone mass, strength and osteoporosis in 2010.¹ Bone strength is reflected through bone density and quality. While bone quality refers to the architecture, turnover, damage accumulation and mineralization, bone density is expressed as grams of mineral per area or volume and in any given individual, it is determined by peak bone mass and amount of bone loss.² Peak bone mass is an important determinant of osteoporotic fracture risk and nutritional intake is a critical factor impacting bone maturation and modelling, thus achieve a healthy peak bone mass.^{3,4} It is suggested that in adults and between sexes the BMC and BMD differences are related to greater cortical thickness or width of the bone despite matching body size.⁵ Several studies have indicated that a correlation between BMD and lean body mass may be an influencing factor over the mechanostat set-point leading to bone quality and size differences between sexes.⁶⁻⁹ Fracture in bones with low mineral density or osteoporosis occurs when a failure-inducing force is applied to it. Gender influences over fracture susceptibility is related to greater bone mass and skeletal integrity in males.⁵

Oral implication to low bone mineral density and osteoporosis commonly noted is thinning of the mandibular cortex. While cortical bone cannot be adequately evaluated at the anterior margin of ramus and in maxilla, the alveolar crest residual ridge and alveolar bone density show a significant relationship to total body calcium and regional bone loss.¹⁰ Recent studies show alteration of trabecular bone as a result of low bone mineral density and osteoporosis in dental radiographs,¹¹ while a marked porosity and erosion of mandibular cortex especially below the mental foramen has been strongly associated with reports of low bone mineral content and osteoporotic fractures.¹² Mandibular trauma fractures with highest incidences are seen in the sites of parasymphysis, body, condyle and angle.^{13,14} Low bone mineral density and osteoporosis may also pose a risk to alveolar bone trauma fractures, successful dental implant and partial and complete denture treatments.^{12,15-17}

Current FDA-approved osteoporosis medications include bisphosphonate based drugs and estrogen therapy. Bisphosphonates are useful in decreasing the risk of fractures in individuals who have already sustained osteoporosis related fractures, with the requirement to reevaluate duration of use periodically.^{18,19} Bisphosphonates are associated with concerns of osteonecrosis of jaw and atypical femoral fractures.^{20,21} With little evidence towards benefit and indication of associated potential adverse effects, it is suggested that when used more than three to five years it may be appropriate to stop treatment in some low-moderate fracture risk patients.¹⁹ Estrogen therapy for at least seven years has been indicated for long term preservation of bone mineral density among women 75 years of age and older where the risk of osteoporosis related fracture is highest,²² although data suggests long term past use modestly increases risk of breast cancer.²³ Testosterone therapy has also been shown to improve bone quantity and quality in hypogonadal men but the associated androgenic effects limit their benefits in older patients.^{24,25}

One of the prescribed treatments for osteoporosis includes calcium and vitamin D supplementation.²⁶ However, Ca supplementation by itself did not appear to slow the rapid

loss of trabecular bone during the first few years of menopause, nor did it prevent the menopause related lumbar bone loss.^{26,27} Other trace minerals are also known to be important in maintaining bone quality due to their involvement in synthesis of collagen and other proteins. Magnesium (Mg) deficiency leads to bone loss in what might include a phosphate (P)- induced release of inflammatory cytokines and impaired 1,25-dihydroxyvitamin D and parathyroid hormone.²⁸ Zinc (Zn) is also involved in the bone turnover process by regulating calcitonin secretion, in turn reducing osteoclastic activity, collagen synthesis and alkaline phosphatase activity.^{29,30} Many drugs have been developed to prevent and retard the onset of loss of bone mineral and apatite content by reducing the tendency for increased bone resorption with age. However, treatments are not currently available to regenerate bone that has already been lost.

Multiple studies in the past have studied the effects of mineral deficiency, low BMD, BMC and osteoporosis, mechanisms and therapeutics in FDA-approved rat models. However, there is a lack of study in the field of evaluating peak bone mass in mandible, consequent effects and susceptibility to trauma as a result of low BMD and BMC. This study aims to test the research hypothesis that a novel calcium phosphate based synthetic bone mineral material (SBM), used as a dietary supplement, would have a long-term preventive and therapeutic effect in preserving and promoting mandibular bone formation in male and female rats with mineral deficiency. Relative to the BD, MD would reduce the bone mineral volume, density and increase porosity.

MATERIALS AND METHODS

Preparation of SBM

The experimental SBM was prepared by a modified hydrolysis method.^{31,32} Briefly, the mixture of dicalcium phosphate dihydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, Mg and Zn chlorides (MgCl_2 , ZnCl_2 respectively) was hydrolyzed in double distilled water containing dissolved potassium carbonate. Reaction temperature was 95°C; pH unadjusted and reaction time 16 hours.

The SBM preparations were characterized using X-ray diffraction (XRD) (Philips X'Pert), Fourier Transform Infrared spectroscopy (FTIR) (Nicolet Magna IR 550), Inductive coupled plasma (ICP) (Thermo Jarrell Ash, Trace Scan Advantage) and thermal analysis; DSC-TGA (SDT Q600, Texas Instruments).

Bone composition and mineral properties

FTIR—Bones are composed of organic- predominantly collagen and inorganic-mineral-carbonate apatite phases. FTIR was used to calculate the relative organic/inorganic ratios qualitatively from ratios of the intensity of absorption band at about 1660 cm^{-1} attributed to the amide N-H group in the collagen and intensity of the strongest absorption band at around 1045 cm^{-1} of the PO_4 of the carbonate apatite.³³

Thermal analysis—DSC-TGA instrument was used to quantify adsorbed water, organic and carbonate content from weight loss in the sample at different ranges of temperature. The mineral content is the remaining weight plus the carbonate content. 10 mg bone powder was heated from room temperature (25°C) to 950°C at a rate of 20°C/min.³³

ICP—Composition of Ca, P, Mg and Zn in the bone was determined using ICP instrument. 10 mg of powdered bone mineral; ashed at 600°C, was dissolved in 1:1 HCl, and made up to 100 ml (with doubled distilled water) in a volumetric flask. Appropriate standard solutions for Ca (0, 20, and 40ppm); P (0, 10, and 20 ppm); Mg (0, 1, and 10 ppm); Zn (0, 1, and 10 ppm); Na (0, 1, and 10 ppm) and K (0, 1 and 10 ppm) were prepared from 100 ppm standard solutions of the respective elements (Fisher Scientific). The specimen in solution and standard solutions were pumped through argon plasma excited by 2 KW 27.12 MHz radio frequency generators. The characteristic wavelengths for the elements determined were: Ca: 317.9 Å; P: 213.6 Å; Mg: 279.5 Å; Zn: 202.5 Å; Na: 588.9 Å; and K: 766.4 Å.³³

XRD—Finally, the crystallite size of the bone mineral was determined using XRD (phillips X'per X-rat diffractometer) using curved crystal monochromator, operating at 45 KV and 45mA, scanning in the range of 20–40°, 0.02°(2θ) step size at 3 sec/step. Crystallite size along the c-axis direction was determined from the broadening at half height ($\beta_{1/2}$) of the [002] diffraction peak (at around 25.8° 2θ) using the Scherrer formula: $t = 0.9\lambda / (\beta_{1/2} \cos\theta)$, where 't' is crystallite size, λ (wavelength) = 1.545Å, θ = diffraction angle, $\beta_{1/2}$ is the difference of sample and instrumental broadening, $\beta_{1/2} = (B2 - b2)^{1/2}$, B2 = observed broadening from the XRD pattern, b2 = instrumental broadening (0.001 radian) and $\beta_{1/2}$ is the difference of sample.³³

Animal experiments

Animal diet—The BD, MD and MD+SBM diets were prepared by Purina Test Labs (Indiana). In comparison to previous studies, the dosage of SBM was increased to 2% in this study. The mineral composition (in wt%) of the diets were as following:

Basic diet: Ca,0.6; P,0.57; Mg,0.07; Zn,21 ppm; Na,0.21 and K,0.4.

Mineral deficient (MD): Ca,0.0; P,0.17; Mg,0.0; Zn,0 ppm; Na,0.0; K, 0.4

MD + 2% SBM (123S): Ca,0.50; P,0.37; Mg,0.05; Zn,529 ppm; Na,0.15; K, 0.37

Animals—Sixty, Sprague-Dawley rats were obtained from Charles River Laboratories, USA, with body weights for females measured between 230 – 280 g and males 450 – 525 g. They were randomly divided into 6 groups (10 rats per group). Group 1 (F1) consisted of females on BD. Group 2 (F2), females on MD. Group 3 (F3), females on test diet- MD +SBM. Group 4 (M1), Males on BD. Group 5 (M2), Males on MD. Group 6 (M3), Males on test diet- MD+SBM.

The rat model utilized here follows life span comparisons common to that of all laboratory rats, with a life expectancy averaging 2 – 3.5 years compared to a worldwide human average 66.7 years.³⁴ This study was started with rats aged 6 months, representing an age of social maturity, while senescence is achieved in female rats between 15 – 24 months of age³⁴ and beyond 10 months of age rats are considered skeletally mature³⁵. During this phase of rat life 11.8 rats days would equate to 1 human year.³⁴

Animal care—The animal protocol was approved by the NYU institutional animal care and use committee (IUCAC) and NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. The 6-month-old rats were housed in same sex pairs in cages at the New York University College of Dentistry under regulated light/dark illumination cycle with constant temperature and humidity. The rats were pair fed; given non-fluoridated water *ad libitum*, monitored daily and their weights and their weights recorded weekly. Each diet group consisted of 5 cages (2 rats per cage) and each cage was given 240 g of food every 4 days. The remaining food at the beginning of next feed cycle was weighed and removed before replacing with fresh stock.

After a period of 6 months they were sacrificed by CO₂ inhalation, utilizing a CO₂ chamber (Euthanex, USA). The rats were exposed to a gas mixture of 30% CO₂ and 70% O₂ for 2 minutes followed by 100% CO₂ for 4 minutes at a flow rate of 25 psi until no sign of breathing was detected. Thereafter, their mandibles were isolated, soft tissues resected and bones stored in 70% alcohol at -20°C. The hemi-mandibles were then separated at the symphysis, incisors removed and stored in 70% alcohol.

Analysis of bone mineral density and microarchitecture

Micro-computed tomography (μCT)—μCT has been used extensively to quantify the 3-dimensional microarchitecture of trabecular bone.^{36–38} The hemi-mandibles in 70% alcohol were placed in the μCT specimen holder and sealed using styrofoam blocks to stabilize the bones. Bone mineral volume and microarchitecture of mandibular body and condyle were evaluated using the μCT (μCT 40, Scanco Medical, Basserdorf) with a resolution of 20 μm. One hundred and fifty μCT slices were imaged over the areas of interest using 55kVp energy and 145 μA current. The integration time utilized was 200 ms and total scanning time of 21.1 minutes. The μCT slices were images which were filtered using a constrained three-dimensional Gaussian filter and binarized using a fixed threshold of 240. 3-D construction of the condylar cross sections were made with a reference point set approximately 100 slices from condyle tip and body of mandible, 25 slices mesial to the first molar. The 3D structural parameters (BV/TV and porosity) were obtained using Sigma 1.2, Support 1.0, software provided by the manufacturer.

Microradiography (Faxitron)—The left and right quadrants of the mandible separated at symphysis were examined using X-ray radiography; X-ray system faxitron series (43805N), Hewlett Packard, using consistent settings 30 kV and 2.5 mA for 18 sec utilizing dental occlusal films (Kodak insight 10–41). Films were processed and scanned together using a Gendex (Lake Zurich, IL) GXP dental X-ray processor and a Minolta Dimage Scan Dual II (Konica, New York, NY). Graded aluminum wedges of densities 1.1849 g/cm³ (0.02 mm x 80 folds), 1.14611 g/cm³ (0.02 x 60 folds), 1.0414 g/cm³ (0.02mm x 40 folds), 0.9346 g/cm³ (0.02mm x 30 folds) and 0.759447 g/cm³ (0.02mm x 20 folds) g/cm³ were placed adjacent to the bone for gray-scale standardization. Gray-scale values of the standard Al wedges and the areas of interest in mandible were obtained using BioQuant Osteo Version 7.10.10 MR.³⁹ Average values and standard deviations were obtained for alveolar bone density (ABD) and alveolar crest density (ACD), all of which are associated with bone resorption in different areas of the mandible.⁴⁰ The desired region for evaluation was first marked over the

digitized radiograph and the gray-scale value obtained. The density calculations were then made using the gray-scale or intensity values through a standard equation.³⁹ The mass absorption coefficient of aluminum for copper K-alpha and copper K-beta x-ray radiation is described in the Handbook for Chemistry and Physics.⁴¹ The mass absorption of coefficients of actual bone elements (excluding the organic but including the mineral) e.g. Ca, P, Mg, Zn etc. can be determined and added together.

The bone density is determined through the brightness of Faxitron image to equivalent thickness of the aluminum and then correcting for the ratio of bone sum of mass absorption coefficient to the aluminum mass absorption coefficients for the x-ray copper K-alpha and copper K-beta radiation. The radiographic vertical bone loss measurements employed in this study are believed to be more sensitive than horizontal bone loss measurements.⁴⁰

Statistical analysis

The research hypothesis of this study was that relative to the BD, MD would reduce the bone mineral volume, density and increase porosity, while SBM would preserve, improve and promote bone formation. 2-way ANOVA was used to compare mean effects of 3 diets and 2 gender groups. Parallel analyses of dependent variables were made: BV/TV-bone volume (% values); Porosity (% values); Alveolar bone density (g/cm^3) and Alveolar crest density (g/cm^3) in the mandibular body, condyle and alveolar regions. The mean $\pm$ SD were obtained along with p values, homogeneity tests, estimates of effect size and observed power, for a pre-determined significance level 0.05 and 95% CI. A graph representing the mean and 95% CI was derived for each set of data. Data analyses were made using IBM, SPSS (v22; IBM Corp., Armonk, NY).

RESULTS

Characterization of SBM and rat bone

Normal rat bone mineral and SBM were found to have similar apatitic structures when analyzed through FTIR and XRD (Figure 1). The FTIR spectra showed similarities in the P-O absorption bands and C-O absorption bands (for the CO_3 groups) in apatite characteristic of carbonate substituted apatite or carbonate apatite (Figure 1D and 1E). Table 1 shows the composition (wt.%) and crystal sizes of rat bone mineral and SBM.

Analysis of ashed bones from SBM rats revealed higher Mg^{2+} and Zn^{2+} concentration than that seen in the bones of rats on MD (Table 2). This suggests that the SBM was incorporated in the bones of the rats on diet supplemented with SBM, consistent with previous studies.³³

During thermal analysis, the weight lost between room temperature and 120°C represented adsorbed water; between 120 and 400°C ; organic matter and between 500 to 950°C CO_3 content. This last fraction plus the remaining weight represented the total mineral content. The ratio of organic to inorganic weight was calculated by dividing the weight loss between 120 to 400°C by the mineral content.

Animal body weight, health and diet consumed

There was comparable body weight gain in male and female rats throughout the study [M(SD)] (Figure 2C). Although the same quantity of food was provided to all rats, males consumed significantly more food than the females and gained more weight (Figure 2A and 2B).

Effects on bone density

Compared to the rats on BD and supplemented diet, the microradiographs in Figure 3 show a marked increase in translucency (indicating bone loss) in the rats on MD. Alveolar bone loss was also visible in transverse 3D and 2D μ CT reconstructions of the same area of the mandible (Figure 4), where the alveolar crest and incisor bone socket were prominently affected. Figure 6A suggests that ABD was more affected by diet in males than in females. Analysis showed an interaction ($p = 0.013$) indicating that males experienced almost twice the decrease in bone density on the MD diet (4.19%) as the females (2.35%). By contrast, the SBM diet preserved bone density similarly in both genders (+0.6% in females and +0.4% in males). Thus the MD resulted in reduced alveolar bone density, more in males than females, and the SBM reversed this effect, similarly in both genders.

Compared to rats on BD and SBM, the microradiographs in Figure 3 also show a marked increase in translucency of alveolar crest bone in the rats on mineral deficient diets. Figure 6B suggests that mean ACD (g/cm^3) was differently affected by diet in the males than the females. Analysis supported this interaction ($p < 0.001$), indicating a strong decrease in ACD in males (3.3%) but not female (0.7%) on MD. Further, while the SBM diet preserved crest density in MD males, it increased ($p < 0.05$) crest density in the females (+0.8% in females vs +0.3% in males). Thus, the mineral deficient diet resulted in reduced alveolar crest density in males but not females, and the supplemental diet reversed this effect in the males and increased density in the females.

Effects on bone mineral volume and microarchitecture

The μ CT evaluation suggests greater deterioration in the trabecular bone in females than males, and greater deterioration in the cortical bone in males than females (Figure 5). Analysis of the data from body of mandible Figure 5B showed volume loss and increase in porosity in male MD rats SBM maintained bone volume and reduced porosity in the males and increased it in females ($p < 0.001$). These results were similar to those seen in the alveolar crest (Figure 6C and 6D). That is, the MD diet resulted in more porosity and lesser bone volume in males but not females, and the supplemental diet reversed this effect in the males, and led to reduced porosity and higher BV/TV in the females.

Compared to rats on basic diet and supplemented diet, the μ CT images of condyle in Figure 5A also show a marked increase in porosity and decrease in BV/TV in the rats on MD diets. Figure 6E and 6F suggest that diet affects bone volume and porosity similarly in the two genders, although measures suggested higher bone volume in the males. Analysis supported this observation, showing main effects of diet ($p = 0.013$) and gender ($p < 0.001$) on porosity and bone volume [diet ($p = 0.009$) and gender ($p < 0.001$)]. That is males showed less porosity than females on SBM [M(SD) = 82.7%(1.1) and 85.1%(1.3), respectively] and the

MD increased porosity similarly for both genders [M(SD) = 83.9%(1.1) and 86.0%(0.4), respectively]. In a similar way, males showed more bone volume than females [M(SD) = 17.0%(1.2) and 15.2%(1.2), respectively] and the MD decreased bone volume similarly for both genders [M(SD) = 16.0%(1.1) and 13.9%(0.4), respectively]. Thus, the pattern of effects on the condyle was different than other regions- MD increased porosity and decreased bone volume and SBM reversed this effect similarly in males and females.

DISCUSSION

Across the mandible, bone quality varied with region, gender and diet. In the alveolar bone, MD reduced BMD significantly more in males than females, and SBM preserved BMD in both. For the alveolar crest, MD significantly reduced BMD only in the males, and SBM preserved male bone and significantly supplemented female bone. For mandibular body, similar to the alveolar crest, MD significantly increased porosity and reduced bone volume only in the males, and SBM preserved male bone and significantly supplemented female bone. For the condyle, MD significantly reduced while SBM preserved BMC similarly in males and females.

Previous studies have demonstrated significant effects of various SBM preparations on improving bone strength and therapeutically improving bone quality in estrogen deficient, ovariectomized and osteoporotic animal models.^{33,39,42–47} This study evaluated the efficacy of SBM as a preventive supplement for long-term preservation and promotion of bone formation in the mandible with low BMC and BMD, as a result of impairment in peak bone mass acquisition regardless of gender. Peak bone mass is a vital factor in achieving healthy bone quality^{2,3} while nutrition and exercise are important in achieving a healthy peak bone mass.^{48–50} Physical activity and testosterone are seen to be correlated with increased lean muscle mass, BMD and bone size,^{51–53} although gender differences in BMD may be site-dependent to areas that have a large proportion of cortical bone.⁵ Since the bone maturation period is longer in males than females, bone size and cortical thickness is hence larger in males, while no significant differences between sexes is noted in trabecular density.⁵⁴ Bone loss may also increase with age and in individuals who do not reach peak bone mass during childhood and adolescence.²

This study thus utilized young male and female rats, which had not reached their peak bone mass, to evaluate how MD affected bone quality and density in the mandible and the efficacy of SBM in preserving existing bone from negative turnover and promoting bone formation in growing rats compared to MD and BD rats. The mandible and alveolar bone proper is composed primarily of compact bone (cortical bone) while the supporting alveolar bone contains trabecular bone between the lingual and facial plate and the alveolar bone proper.⁵⁵ The male rats in our study achieved a more impaired peak bone mass compared to females thus the MD renders more effects in cortical bone of males. Although difficult to ascertain in male rats, female rats show peak bone density at around 9–10 months.^{56,57}

MD diet accelerated bone loss, increased bone porosity and decreased density of alveolar bone, condyle and body of mandible. It can thus be established that nutritional deficiency leads to low BMC and BMD, while SBM prevented the effects of MD diet and improved

bone quality in adult rats with low peak bone mass. More importantly, the study shows that SBM used as a long-term dietary supplement prevents bone loss, preserves and promotes bone formation in the mandible as shown by the improved bone architecture, volume and density of treated groups compared to mineral deficient groups and also BD group. Hence, SBM may be a vital supplement in promoting bone health in adults who are diagnosed with low BMC and BMD, and in young and adolescent individuals who are at a risk for mineral deficiency.

Our earlier studies have shown that Ca-P based compounds (carbonate apatite), incorporating Mg and Zn, were effective in preventing loss in bone strength and bone density. The SBM preparation used in this study consisted of a carbonate apatite (like the bone mineral) incorporating Mg and Zn. These ions have separately been shown to affect bone cell activity in vivo and in vitro. In vitro, Zn ions incorporated in an organic compound⁵⁸ and in an inorganic compound, tricalcium phosphate have been shown to stimulate bone formation in vivo. In vitro, Zn ions in Zn-substituted β -TCP were shown to suppress osteoclastic activity.⁵⁹ Mg ions in Mg-substituted tricalcium phosphate have been shown to promote osteoblastic activity.⁶⁰ However its deficiency has been linked to osteoporosis through alteration of apatite crystals structure, association with phosphate induced release of inflammatory cytokines and impaired 1,25-dihydroxyvitamin D and parathyroid hormone.²⁸ Materials characterization performed in this study indicated that bone composition of rats on MD diet supplemented with SBM showed higher concentrations of Mg and Zn ions compared to those in bones of rats not receiving SBM supplement. This indicates that the Mg and Zn ions incorporated in the SBM were also incorporated in the bone since these elements released from the SBM are bone-seeking elements.^{33,39} Higher concentrations of Mg^{2+} and Zn^{2+} were added to this SBM preparation to take advantage of their known individual positive effects on mineralization and bone cell activity.^{28,30,59} Previous studies also incorporated F ions, but it has been noted that they have limited impact on improving bone quality. Fluoride is present in the drinking water in many countries thus adding F ions to our supplement may increase its consumption.⁶¹

The current study also evaluated differences in bone quality and density between sexes. An important observation made through this study indicated that bone quality in supplemented male models improved significantly more than females. This can be implicated to higher intake of diet in males. Eating more not only meant higher uptake of minerals but also higher lean muscle activity related to the mandible which leads to improvement in BMC and BMD, also noted earlier.^{51–53} Males have higher bite force and muscular activity related to mandibular activity.⁶² At the end of the study, relatively higher BMC and BMD was noted in male SBM and BD groups since rats were allowed to achieve high peak bone mass, with SBM group higher than BD. When compared to BD model the MD male rats showed relatively lower BV/TV and BMD values in cortical bone since they achieved a lower peak bone mass through mineral deficiency. We can hypothesize that mineral sufficiency before and during the process of achieving peak bone mass is vital in maintaining healthy bone turnover.

Traditionally low bone mass (high turnover) in the femoral neck or lumbar spine is an important indicator to osteoporosis risk.⁶³ The mandibular bone and attached muscles are

exposed to daily physical activity; involving speaking, chewing, swallowing, thus constant bone turnover is natural. Through this study, we also indicate that mandibular evaluation in the body, condyle and alveolar region may be used as indicators to low BMD and BMC as a result of low peak bone mass.

Current osteoporosis therapeutics have been shown to prevent additional bone loss but have not been shown to restore bone already lost from the disease, and are reported to be associated with serious side effects. With rising health care costs, increased incidence of fractures due to bone loss, and safety concerns associated with the side effects from current therapies, additional safe, affordable and efficacious treatment options are needed for osteoporosis. In the future, more aggressive treatments for low bone mineral density and osteoporosis will likely rely on the activation and reactivation of bone metabolic functions, and the development of new drugs or supplements that will activate and enhance bone formation and bone metabolism without causing adverse side effects.^{64–68} The present investigation supports the potential of SBM as an effective biomaterial for the prevention osteoporosis in the presence of mineral deficiency. The results of this study suggest that SBM will prevent low BMD, BMC, osteoporosis and/or other diseases associated with MD and has a potential to do this more safely than current treatments. Current studies in our lab are investigating the preventive and recovery benefits of SBM in estrogen-deficient rats, on other skeletal locations of the body, on improving mechanical properties of bone and analyzing SBM as a bone grafting biomaterial.

In summary, we confirm the research hypothesis. Compared to BD, MD lead to impairment of peak bone mass acquisition as a consequence of nutritional deficiency, affecting the alveolar bone, body of mandible and condyle, more in the males than females. SBM administered orally as a supplement prevented mandibular bone loss across both genders and promoted bone formation in female rats.

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Fig. 1. Characterization of SBM and rat bone. (A) TGA analysis of rat bone. XRD profiles of (B) rat bone mineral and (C) synthetic bone mineral (SBM), showing 'apatitic' structure with similar crystallinity. FT-IR spectra of (D) rat bone mineral and (E) SBM, showing characteristic IR absorption bands of carbonate apatite.

Fig. 2.

Animal weight gain (A) and diet consumed (B) across diet and gender groups, over 6 months study period. Values are mean $\pm$ SD (C). F1 (females on basic diet), F2 (females on mineral deficient diet), F3 (females on mineral deficient diet with 2% SBM); Group 2: M1 (males basic diet), M2 (males mineral deficient), M3 (males mineral deficient with 2% SBM).

Fig. 3.

High resolution radiography showing differences in density in the alveolar bone and alveolar crest regions (circled). F1 (females on basic diet), F2 (females on mineral deficient diet), F3 (females on mineral deficient diet with 2% SBM); Group 2: M1 (males basic diet), M2 (males mineral deficient), M3 (males mineral deficient with 2% SBM).

Fig. 4.

3D Micro-CT transverse section images of the left hemi-mandible (left) and 2D high resolution radiographic images (right) of corresponding cross-sections, showing differences in density in the alveolar bone (3D arrows; 2D circled). Compared to rats on basic diet (female-F1, male-M1) bone loss is evident in mineral deficient rats (female-F2, male-M2) and prevention is apparent in supplemented groups (female-F3 and male-M3).

Fig. 5.

Mandibular areas of interest (Top); Micro-CT images of the condyle (A), body of mandibular bone (B). Compared to female rats on basic diet (AF1, BF1), mineral deficient rats showed bone loss and increased porosity (AF2, BF2) and bone loss prevention and decreased porosity in rats supplemented with SBM (AF3, BF3). Male mineral deficient model showed bone loss and increased porosity (AM2, BM2) and bone loss prevention and decreased porosity in rats supplemented with SBM (AM3, BM3) compared to male rats on basic diet (AM1, BM1).

Fig. 6.

Graphs showing differences in - BV/TV, bone mineral density and porosity between male and female rats on basic, mineral deficient and SBM supplemented diets. Effect of diet and gender on alveolar crest density (A), alveolar bone density (B), body of the mandible- BV/TV (C), body of mandible- porosity(D), condyle- BV/TV (E), condyle- porosity (F).

Note: Bars in gray represent BD, white- MD and black- MD+SBM. Error bars represent the 95% confidence interval (CI).

Table 1

Comparison of rat bone and SBM composition (wt.%) and crystallite size.

	Ca	P	Mg	Zn	F	CO ₃	Na	K	Crystal size
Rat bone	26.08	16.64	0.95	0.08	0.0	4.78	1.38	0.49	272 Å
SBM	25.60	15.03	2.05	1.32	0.0	4.95	0.01	1.47	262 Å

Table 2

Composition of ashed rat bone (wt.%). BD (Basic diet), MD (Mineral deficient diet), MD+2%SBM (Mineral deficient diet with 2% SBM)

	Ca	P	Mg	Zn
BD	36.97 ± 1.4	17.45 ± 0.8	0.85 ± 0.04	0.06 ± 0.01
MD	35.91 ± 2.4	17.40 ± 0.9	0.39 ± 0.08	0.05 ± 0.01
MD + 2%SBM	37.20 ± 1.8	17.93 ± 0.7	0.74 ± 0.00	0.11 ± 0.01